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WASHINGTON, DC 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

June 18, 2002

MEMORANDUM

SUBJECT: Tolylfluanid - Report of the Cancer Assessment Review Committee

FROM: Jessica Kidwell
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Guruva Reddy, Toxicologist
Registration Action Branch 1
Health Effects Division (7509C)

Jennifer R. Tyler, Risk Assessor
Registration Action Branch 1
Health Effects Division (7509C)

Lisa Jones, Product Manager
Registration Division (7505C)

The Cancer Assessment Review Committee met on May 1, 2002 to evaluate the carcinogenic potential of Tolylfluanid. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

TOLYLFLUANID

P.C. Code: 309200

FINAL REPORT
June 18, 2002
TXR NO. 0050810

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

TOLYLFLUANID CANCER ASSESSMENT DOCUMENT FINAL REPORT

DATA PRESENTATION: Guruva B. Reddy, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam

Karl Baetcke

Marion Copley

Vicki Dellarco

Virginia Dobozy

Nancy McCarroll

Tim McMahon

Linda Taylor

Esther Rinde DID NOT ATTEND MEETING

Jess Rowland

Joycelyn Stewart

Clark Swentzel

Yintak-Woo See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively).

John M. Pletcher, Pathology Consultant See attached sheet

Lori Brunsman, Statistical Analysis

This meeting was attended by Pam Hurley /HED, Lisa Jones/RD and John Hancock/Pesticide Management Regulatory Agency (PMRA), Health Canada, Canada.

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** TOTAL PAGE.001 **

TABLE OF CONTENTS

EXECUTIVE SUMMARY

On May 1, 2002 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of tolylfluanid. This meeting was held jointly by teleconference with Pest Management Regulatory Agency, Health Canada, Canada. The long-term dietary studies evaluated included two combined chronic toxicity/carcinogenicity studies in Wistar rats and a carcinogenicity study in $B6C3F_1$ mice.

In a 1982 rat study, 50 Wistar rats/sex/dose received tolylfluanid at concentrations of 0, 300, 1500 and 7500 ppm (0, 20, 80, and 430 mg/kg/day for males and 0, 20, 110, and 580 mg/kg/day for females, respectively) for 24 months. In a 1996 rat study, 50 Wistar rats/sex/dose received the test compound at concentrations of 0, 60, 300, 1500, and 7500 (0, 3.6, 18.1, 90.1 and 504.2 mg/kg/day in males and 0, 4.2, 21.1, 105.2, and 584.4 mg/kg/day in females, respectively) for 105-107 weeks. In a carcinogenicity study in mice, tolylfluanid was administered to groups of 50 male and 50 female $B6C3F_1$ mice at concentrations of 0, 60, 300, 1500 and 7500 ppm (0, 15, 76.3, 375.8 and 2307.6 mg/kg/day for males and 0, 24.5, 123.9, 610.8 and 2962.8 mg/kg/day for females, respectively) for up to 108 weeks.

The CARC concluded that tolylfluanid was carcinogenic to male and female rats.

- In the 1982 study, male rats had a statistically significant increasing trend for combined follicular cell adenomas/carcinomas of the thyroid. The incidence at the high dose was above that of concurrent control and was towards the high end of the historical control range. For females, there was a significant increasing trend for follicular cell adenomas and combined adenomas/carcinomas of thyroid. The incidence of adenomas at the high dose was outside the historical control range. In both male and female rats, the increase in the combined incidence at the high dose was driven by adenomas. The high dose females also developed increased incidence of benign ovarian granulosa-theca cell tumors. Although there was a statistically significant positive trend, the increase in ovarian tumors at the high dose was not statistically significant by pair wise comparison with the controls. There was an increase in the incidence of malignant endometrial stromal sarcomas at the high dose and uterine adenocarcinomas, carcinomas and combined adenocarcinomas/carcinomas at the two top doses. The increase in the combined adenocarcinomas/carcinomas, although statistically significant by pair wise comparison with the controls, showed no positive trend and no-dose response was evident. With the exception of combined uterine adenocarcinomas/ carcinomas, the incidences of the above tumors exceeded their respective historical control range. The CARC determined that the findings of ovarian and uterine tumors did not add to the overall weight-of-the evidence for the carcinogenicity of tolylfluanid because these tumors were not reproducible in the

1996 study.

- In the 1996 rat study, there was a statistically significant positive trend and statistically significant increase by pair wise comparison with the controls for thyroid follicular cell adenomas and combined adenomas/carcinomas in both high dose male and female rats. The statistically significant increases in thyroid follicular cell adenomas and combined adenomas/carcinomas seen in the earlier study were reproducible in this study. The increase in combined incidence in both male and female rats was driven by the adenomas; the tumor incidences exceeded the historical control values. No increases in the ovarian and uterine tumors were noted in this study.

  The highest dose was considered to be adequate and not excessive in both studies based on the following findings in one or both studies: decrease in body weight gain, histopathological changes in the liver, bile duct, thyroid and hyperostosis of the cranium. **The CARC considered the thyroid tumors in high dose male and female rats to be treatment-related.** The Committee also reviewed the *in vitro* and *in vivo* mechanistic studies using the criteria for evaluating evidence for antithyroid activity. It was concluded that the available mechanistic data are insufficient to determine whether the thyroid tumors in rats associated with administration of tolylfluanid are due to a disruption in the thyroid-pituitary homeostasis.

- Tolylfluanid was not carcinogenic to male and female $B_6C_3F_1$ mice.

Tolylfluanid has been tested in *in vitro* and *in vivo* mutagenicity assays and found to induce gene mutations and chromosomal aberrations in mammalian cells. However, this mutagenic activity was not expressed in *in vivo* tests for gene mutations or chromosomal aberrations. In addition, there was no indication of DNA reactivity *in vitro* or *in vivo*. Therefore, the overall weight of the evidence does not support a mutagenic mode of action for the induction of thyroid tumors in rats. The CARC, however, recommended conducting a bone marrow cytogenetic assay in order to reach definitive conclusions regarding the clastogenic potential of tolylfluanid in whole animal somatic cells.

Unlike captan and folpet which are structurally-related compounds, tolylfluanid is not a direct-acting alkylating agent. This is consistent with the much weaker or marginal genotoxic activity of tolylfluanid. Moreover, captan and folpet cause gastrointestinal tumors while tolylfluanid causes thyroid tumors in rats.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified tolylfluanid into the category "**likely to be carcinogenic to humans**" by the oral route. The Committee further recommended using a linear low-dose extrapolation approach

for the quantification of human cancer risk based on thyroid tumors in rats since the mode of action data on these tumors were not adequate to depart from the linear method of risk assessment.

I. INTRODUCTION

On May 1, 2002, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of tolylfluanid. Tolylfluanid has not been previously reviewed by the CARC for its carcinogenicity. This review was conducted jointly with PMRA, Canada. Dr. Guruva Reddy, Registration Action Branch 1/HED, presented the chronic/carcinogenicity studies in Wistar rats and B6C3F$_1$ mice by: describing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested; and presenting the mechanistic studies to support the non-linear mode of action for the carcinogenicity of tolylfluanid. Dr. Reddy also discussed the toxicology, metabolism and mutagenicity studies as well as structure-activity relationships of the related compounds.

II. BACKGROUND INFORMATION

Tolylfluanid (PC. Code: 309200; CAS # 731-27-1.) is a new broad spectrum contact fungicide It is currently registered for use on apples, grapes and tomatoes in several European countries. The use patterns vary depending on crop and country. It has a greenhouse use for tomatoes in Belgium and Netherlands and is intended to be registered in the United States for import tolerance only.

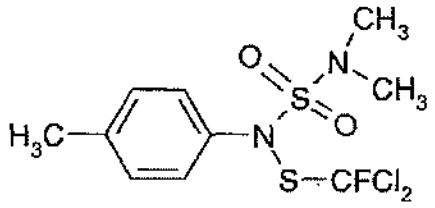


Figure 1. Structure of tolylfluanid

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Tolylfluanid in Wistar Rats (1982 Study)

Reference: Krötlinger, F., Löser, E. (1982) Chronic Toxicological Study in Rats. Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straße, Wuppertal, Germany. Laboratory Study No. T7002834, Report #10978, June 30, 1982. MRID 44247602. Unpublished.

A. Experimental Design

Tolylfluanid (98.8% a.i., Lot # 16001/76, F1.776/121) was administered to groups of 50 male and 50 female Wistar rats in the diet at concentrations of 0, 300, 1500 and 7500 ppm (0, 20, 80, and 430 mg/kg/day for males and 0, 20, 110, and 580 mg/kg/day for females, respectively) for 24 months. Five rats/sex/group were sacrificed at 6 and 12 months for interim evaluations; the remaining animals were maintained on their respective diets for 24 months. Though 100 animals were assigned to the control group for 107 weeks, only the first 50 were examined histologically and included in the analysis.

B. Tumor Analysis and Discussion of Tumor Data

HED's statistical analyses of tumor data in male and female rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. Refer to Tables 1 through 4 for the results of tumor analysis by Brunsman (2002). Male rats had a significant increasing trend for combined thyroid follicular cell adenomas/carcinomas at $p < 0.05$ and there were no significant differences in the pair-wise comparisons of the dosed groups with the controls. The combined incidences of thyroid follicular cell adenomas/carcinomas at 0, 300, 1500 and 7500 ppm were 0/46, 0/50, 0/50, and 3/49, respectively. The increase of 6% for the high-dose group exceeded the control value (0%) and historical control range (0%-6%). There were no carcinomas identified in 30 historical control studies covering a period of 1981 to 1989. Only thyroid follicular cell adenomas were classified.

Female rats had significant increasing trend for thyroid follicular cell adenomas and ovarian granulosa-theca cell tumors, both at $p < 0.05$, and for combined thyroid follicular cell adenomas/carcinomas at $p < 0.01$. The incidences of follicular cell adenomas at 0, 300, 1500 and 7500 ppm were 0/50, 0/50, 0/50 and 3/50, respectively; the incidence of 6% for the high dose group was outside the concurrent control value (0%) and the historical control range of 0%-4% (mean: 0.7%). The combined incidences of thyroid follicular cell adenomas/carcinomas at 0, 300, 1500 and 7500 ppm were 0/50, 0/50, 0/50, and 4/50, respectively. There were no thyroid follicular cell carcinomas identified in 30 historical control studies. The incidences of ovarian granulosa-theca cell tumors in the above dose groups were 1/50, 1/50, 1/50 and 5/50, respectively. The incidence of 10% in the high dose was outside the control value (2%) and the historical control range (performing laboratory: 0%-8%; Bomhard and Rinke, 1994: 0%-8.3%) . In addition, the high dose females had increase in the incidence of stromal sarcomas of the uterus (2/50, 4% vs 0/50, 0% in controls) but the incidence was within the historical control range (performing laboratory: 0%-4%; mean: 0.4%; Bomhard and Rinke; 1994: 0%-6%, 0.6%). There were significant differences in the pair-wise comparisons of the 1500 and 7500 ppm dose groups with the controls for combined uterine adenocarcinomas/carcinomas,

both at $p < 0.05$. The combined incidences of uterine adenocarcinomas/carcinomas were 3/50, 8/50, 11/50 and 10/50 at 0, 300, 1500 and 7500 ppm, respectively; the incidence of 16% for the low-dose, 22% for the mid-dose and 20% for the high-dose were outside the control value of 6% and within the historical control of range of the performing laboratory (0%-24%; man 4.6%) and towards the high end of the historical controls values reported by Bomhard.and Rinke, 1994 (0%-20%; 8%).

Table 1. Tolylfluanid - 1982 Wistar Rat Study

Male Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	300	1500	7500
Adenomas (%)	0/46 (0)	0/50 (0)	0/49 (0)	2a/49 (4)
p =	0.0628	1.0000	1.0000	0.2634
Carcinomas (%)	0/46 (0)	0/50 (0)	0/49 (0)	1b/49 (2)
p =	0.2526	1.0000	1.0000	0.5158
Combined (%)	0/46 (0)	0/50 (0)	0/49 (0)	3/49 (6)
p =	0.0154*	1.0000	1.0000	0.1331

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 107, dose 7500 ppm.
[b]First carcinoma observed at week 107, dose 7500 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Tolylfluanid - 1982 Wistar Rat Study

Female Thyroid Gland Follicular Cell Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	300	1500	7500
Adenomas (%)	0/50 (0)	0/50 (0)	0/50 (0)	3a/50 (6)
p =	0.0149*	1.0000	1.0000	0.1212
Carcinomas (%)	0/50 (0)	0/50 (0)	0/50 (0)	1b/50 (2)
p =	0.2500	1.0000	1.0000	0.5000
Combined (%)	0/50 (0)	0/50 (0)	0/50 (0)	4/50 (8)
p =	0.0036**	1.0000	1.0000	0.0587

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 100, dose 7500 ppm.
[b]First carcinoma observed at week 107, dose 7500 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Tolylfluanid - 1982 Wistar Rat Study

Female Ovarian Tumor Rates[a] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	300	1500	7500
Granulosa-Theca Cell Tumors (%)	1a/50 (2)	1/50 (2)	1/50 (2)	5/50 (10)
p =	0.0161*	0.7525	0.7525	0.1022

[a]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First granulosa-theca cell tumor observed at week 107, dose 0 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Tolylfluanid - 1982 Wistar Rat Study
Female Uterine Tumor Rates* and Exact Trend
Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	300	1500	7500
Endometrial Stromal Sarcomas	0/50	0/50	1/50	2a/50
(%)	(0)	(0)	(2)	(4)
p =	0.0616	1.0000	0.5000	0.2475
Adeno-carcinomas	3/50	6/50	9/50	8b/50
(%)	(6)	(12)	(18)	(16)
p =	0.1579	0.2435	0.0606	0.0999
Carcinomas	0/50	2c/50	2/50	2/50
(%)	(0)	(4)	(4)	(4)
p =	0.2213	0.2475	0.2475	0.2475
Adenocarcinomas and/or Carcinomas Combined	3/50	8/50	11/50	10/50
(%)	(6)	(16)	(22)	(20)
p =	0.1139	0.0999	0.0204*	0.0357*

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First endometrial stromal sarcoma observed at week 98, dose 7500 ppm.

[b]First adenocarcinoma observed at week 81, dose 7500 ppm.

[c]First carcinoma observed at week 81, dose 300 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

Skeletal Lesions

At the final necropsy in the high-dose animals, hyperostosis of the cranium (36/38 males vs 0/40 controls; 20/40 females vs 0/41 controls) and sternum (16/38 males vs 0/40 controls; 12/40 females vs 0/41 controls) were observed. A lengthening of the incisors was noted at the interim necropsy in the high-dose males (3/5 treated vs 0/5 controls) and at the final necropsy, hardening of the cranium was observed in high-dose males (39/50 treated vs 0/100 controls) and females (25/50 treated vs 0/100 controls).

The 1500 ppm males demonstrated hyperostosis of the cranium (3/42 vs 0/40 controls) and sternum (4/42 vs 0/40 controls) at the final necropsy. No additional alterations of toxicological concern were observed in this dose group.

Liver and Other Lesions

Liver necrosis (5/38 treated vs 0/40 controls) was observed in the high-dose males,. In the high-dose females, bile duct proliferation (32/40 treated vs 16/41 controls) and thyroid follicular cysts (8/40 treated vs 0/41 controls) were observed. Other effects observed at 7500 ppm included reductions in body weight (↓10-14%, $p \leq 0.01$) throughout the course of the study, and reduction in overall body weight gains (↓12-16%) and total feed consumption (↓9-14%).

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate and not excessive based on reductions in body weight (↓10-14%, $p \leq 0.01$) throughout the course of the study, and reduction in overall body weight gains (↓12-16%) and total feed consumption (↓9-14%) seen at 7500 ppm. Additionally at necropsy, hyperostosis of the cranium in high-dose males and females, liver necrosis in high-dose males as well as bile duct proliferation and thyroid follicular cysts in the high-dose females were observed. The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of tolylfluanid in male and female rats.

2. Combined Chronic Toxicity/Carcinogenicity Study with Tolylfluanid in Wistar Rats (1996 Study)

Reference: Leser, K.H., Rosenbruch, M., Rinke, M., (1996), Study on chronic toxicity and carcinogenicity in Wistar rats. Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straβe, Wuppertal, Germany. Laboratory Study No. T0040420, September 13, 1996, MRID 44241027. Unpublished.

A. Experimental Design

Tolylfluanid (97.2 to 98.3% a.i., Lot # F1.185) was administered in the diet to Wistar rats (50/sex/dose) at dose levels of 0, 60, 300, 1500, and 7500 (0, 3.6, 18.1, 90.1 and 504.2 mg/kg/day in males and 0, 4.2, 21.1, 105.2, and 584.4 mg/kg/day in females, respectively) for 105-107 weeks. Ten rats/sex/group were sacrificed at 52 weeks for interim evaluations; the remaining animals were maintained on their respective diets for up to 24 months.

B. Tumor Analysis and Discussion of Tumor Data

Refer to Tables 5 and 6 for the tumor analyses results by Brunsman (2002). Male rats had significant increasing trends at $p < 0.01$, and significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls at $p < 0.05$, for thyroid follicular cell adenomas and combined adenomas/carcinomas. The incidences of thyroid follicular cell adenomas at 0, 60, 300, 1500 and 7500 ppm, were 0/49, 1/46, 1/50, 1/46 and 5/50, respectively. The incidence of 10% for the high dose group was outside the control value (0%) and historical control mean of 1.2%. The combined incidences of adenomas/carcinomas in the above dose groups were 0/49, 1/46, 1/50, 1/46 and 6/50, respectively. The incidence of 12% for the high-dose group was outside the control value of 0%. Historical control data from 30 studies did not report occurrence of thyroid follicular cell carcinomas.

Female rats had significant increasing trends at $p < 0.01$, and significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls at $p < 0.05$, for thyroid follicular cell adenomas and combined adenomas/carcinomas. The incidences of thyroid follicular cell adenomas at 0, 60, 300, 1500 and 7500 ppm, were 0/49, 0/48, 0/47, 0/50 and 5/46, respectively. The incidence of 11% for the high-dose females was outside the concurrent control value (0%) and the historical control range of 0%-4% and the mean of 0.7%. The incidences of combined adenomas/carcinomas. in the above dose groups were 0/49, 1/48, 0/47, 0/50 and 5/46, respectively. The incidence of 11% for the high-dose females was outside the control value (0%-4%; mean:0.7%). In 30 historical control studies no thyroid follicular carcinomas were identified.

Table 5. Tolylfluanid - 1996 Wistar Rat Study

Male Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)				
	0	60	300	1500	7500
Adenomas (%)	0/49 (0)	1a/46 (2)	1/50 (2)	1/46 (2)	5/50 (10)
p =	0.0040**	0.4842	0.5051	0.4842	0.0296*
Carcinomas (%)	0/49 (0)	0/46 (0)	0/50 (0)	0/46 (0)	1b/50 (2)
p =	0.2075	1.0000	1.0000	1.0000	0.5051
Combined (%)	0/49 (0)	1/46 (2)	1/50 (2)	1/46 (2)	6/50 (12)
p =	0.0011**	0.4842	0.5051	0.4842	0.0142*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 90, dose 60 ppm.

[b]First carcinoma observed at week 96, dose 7500 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Tolylfluanid - 1996 Wistar Rat Study

Female Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

	Dose (ppm)				
	0	60	300	1500	7500
Adenomas (%)	0/49 (0)	0/48 (0)	0/47 (0)	0/50 (0)	5a/46 (11)
p =	0.0002**	1.0000	1.0000	1.0000	0.0237*
Carcinomas (%)	0/49 (0)	1b/48 (2)	0/47 (0)	0/50 (0)	0/46 (0)
p =	0.4042	0.4948	1.0000	1.0000	1.0000
Combined (%)	0/49 (0)	1/48 (2)	0/47 (0)	0/50 (0)	5/46 (11)
p =	0.0008**	0.4948	1.0000	1.0000	0.0237*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 86, dose 7500 ppm.

[b]First carcinoma observed at week 107, dose 60 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions

Skeletal Lesions

Significantly increased fluoride deposition in the teeth and bone was observed in both sexes. In the teeth, deposition was elevated ($p \leq 0.05$) in both sexes at 53 weeks at doses ≥300 ppm (↑269-2436%,) and at 105-107 weeks at doses ≥1500 ppm (↑335-1165%). In the bone, deposition was elevated ($p \leq 0.05$) at 53 weeks at doses ≥1500 ppm (↑486-1187%) and at 105-107 weeks at doses ≥300 ppm (↑169-1420%).

In the 1500 ppm group at the interim necropsy (52 weeks), altered bone matrix or hyperostosis of the cranium were observed in the females (2/10 treated vs 0/10 controls); at the final necropsy (105-107 weeks), the males had an incomplete demineralization of the osseous matrix of the femur (4/49 treated vs 0/50 controls; p=not significant). No additional treatment-related changes were observed in this dose group.

Statistically significant ($p \leq 0.01$), treatment-related histopathological changes of the bone were also detected in the high-dose animals compared to 0/49-50 controls as follows: osteoporosis of the sternum (41/50 males and 29/48 females), hyperostosis of the cranium (31/50 males and 25/46 females), and thickened osseous matrix of the nasal cavity (14/50 males and 6/48 females). The sternum was also incompletely demineralized in the males (9/50 treated versus 0/50 controls). Additionally, incomplete demineralization of the osseous matrix of the femur was observed in the high-dose males (32/50 versus 0/50 controls).

Liver and Other Lesions

At 7500 ppm, females exhibited changes in both the cytoplasm and the nuclei of hepatocytes (hepatocellular alteration, 35/49 treated vs 1/50 controls, $p \leq 0.01$), hepatocellular vacuolation (38/49 treated vs 7/50 controls, $p \leq 0.01$) and "focal fatty changes" (8/49 treated vs 2/50 controls, $p \leq 0.01$). In males, the periportal liver cells were occasionally hypertrophic (19/50 vs 0/50 controls, $p \leq 0.01$). Effects on the kidneys were demonstrated as minor changes in urine parameters which were associated with slight histological alterations such as an increase in pigment (lipofuscin) deposition in the tubular epithelium (36/49 treated vs 4/50 controls, $p \leq 0.01$) in the females and a slight mineralization of the renal papilla (22/50 treated vs 4/50 controls, $p \leq 0.01$) in the males. Additionally, increased incidences ($p \leq 0.05$ or not significant) of thyroid follicular cell hyperplasia were observed in animals (7/48-50 treated vs 1-2/49-50 controls).

D. Adequacy of the Dosing for the Assessment of Carcinogenicity

Dosing was considered by the CARC to be adequate and not excessive based on decrease in body weights and body weight gains (11%-19%), and changes in bone mineralization in 7500 groups. Significantly increased fluoride deposition in the teeth and bone occurred in

both sexes. Statistically significant ($p \leq 0.01$), treatment-related histopathological changes of the bone were also detected in the high-dose animals. Effects on the kidneys consisted of minor changes in urine parameters associated with a slight increase in lipofuscin deposition in the tubular epithelium in the females and a slight mineralization of the renal papilla in the males. Additionally, increased incidences of thyroid follicular cell hyperplasia were observed in animals. The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of tolylfluanid in male or female rats.

3. Carcinogenicity Study in $B6C3F_1$ Mice

Reference: Leser, K.H. and C. Ruhl-Fehlert (1996) Oncogenicity Study in $B6C3F_1$ Mice. Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straße, Wuppertal, Germany. Laboratory Study No. T8040419, October 17, 1996. MRID 44241028. Unpublished.

A. Experimental Design

Tolylfluanid (97.2 to 98.3% a.i., Lot # F1.185) was administered to groups of 50 male and 50 female $B6C3F_1$ mice in the diet at concentrations of 0, 60, 300, 1500 and 7500 ppm for up to 108 weeks. An additional 9-11 mice/sex/group were sacrificed at 52 weeks for interim evaluation. Time-weighted average doses were 0, 15, 76.3, 375.8 and 2307.6 mg/kg/day for males and 0, 24.5, 123.9, 610.8 and 2962.8 mg/kg/day for females, respectively.

B. Discussion of Tumor Data

A summary of the commonly occurring neoplasm seen at terminal sacrifice in this study is given in Table 7. No significant increases in the incidences of any neoplasms were observed at any treatment level. An increased number of 7500 ppm females, which died intercurrently, were found to have systemic histiocytic sarcoma. However, there was not a significant trend in this lesion when all female animals were considered.

Table 7. Incidence of systemic histiocytic sarcoma in female mice dosed with tolylfluanid for 104-107 weeks.[a,b]

	Dose (ppm)				
Time of Death:	0	60	300	1500	7500
Intercurrent	3	3	2	2	6
Final necropsy	1	2	2	0	0
Total	4	5	4	2	6

[a] These data were extracted from the study report, pages 1002, 1008, and 1014.

[b] 50 animals/dose were examined.

C. Non-neoplastic Lesions

Fluoride content was increased ($p \leq 0.05$) in the teeth and bone in a dose-dependent fashion. In the teeth, fluoride deposition was enhanced in males at doses ≥300 ppm and in females at doses ≥1500 ppm. In the bone, deposition was enhanced at ≥300 ppm in both sexes.

In the 1500 ppm group, hyperostosis of the cranium and elevated alkaline phosphatase levels were detected in males and females. Also, centrilobular hypertrophy and nuclear inclusions (10/50 treated vs 1/50 controls) were observed in males, while peripherolobular hypertrophy was observed in the females. Papillary mineralization of the kidneys was observed in the males (11/50 treated vs 2/50 controls; Table 8).

Boney changes were also observed in the 7500 ppm group as follows: Hyperostosis or hyperostotic lesions of the cranium were observed in 17/47 males and 44/50 females vs 0/50 control males and 2/49 control females. These lesions were also observed in the nose - 9/50 treated males and 43/49 treated females vs 0/49-50 controls. Hyperostotic lesions in females were also observed in the sternum (48/50 treated vs 4/49 controls), femur (17/50 treated vs 8/49 controls), and spinal cord (44/50 treated vs 4/49 controls).

Table 8. Incidence of selected, non-neoplastic, histological findings at final necropsy in mice dosed with tolylfluanid for 104-107 weeks.[a,b]

Dose (ppm)	0	60	300	1500	7500	0	60	300	1500	7500
Sex	Male					Female				
CRANIUM										
Hyperostosis	0	1	1	5	17	0	0	0	0	0
Fibroosseous lesion	0	0	0	0	0	7	3	10	7	0
Hyperostotic lesion	0	0	0	0	0	2	4	6	23	44
NOSE										
Hyperostosis	0	0	0	0	9	0	0	0	0	0
Fibroosseous lesion	0	1	0	0	1	10	21	21	29	1
Hyperostotic lesion	0	0	0	0	0	0	2	1	6	43
STERNUM										
Hyperostotic lesion	0	0	2	0	0	4	5	9	8	48
Fibrosseous lesion	1	0	0	0	0	39	41	38	38	1
FEMUR										
Focal spongiosa	2	4	1	3	4	5	5	8	12	37
SPINAL CORD										
Fibroosseous lesion	0	0	0	0	0	29	33	38	39	5
Hyperostotic lesion	0	0	0	0	0	4	1	3	8	44
LIVER										
Centrilobular hypertrophy	2	2	0	26	46	0	0	0	0	0
Peripherolobular hypertrophy	0	0	0	0	0	4	1	1	14	19
Biliary cystic changes	0	0	1	0	1	1	0	2	1	9
Unicellular necrosis	0	1	0	2	25	0	1	3	1	1
Nuclear inclusions	1	0	0	10	36	0	0	0	0	0
KIDNEYS										
Papillary mineralization	2	1	3	11	34	6	2	7	5	17
Tubular casts	1	2	2	1	11	1	0	4	0	2
Tubular hyperplasia	1	5	6	6	9	1	0	1	0	1
EYES										
Lenticular degeneration	5	7	5	9	31	3	6	8	2	3

D. Adequacy of Dosing for Assessment of Carcinogenicity

The study was conducted above the limit dose (7500 ppm; HDT). The CARC determined that the dosing at the highest dose was adequate and not excessive based on decrease in terminal mean body weights and body weight gains (38%) in high-dose males, and changes in the liver and kidneys of mid- and high-dose animals. Interference with the fluoride metabolism was the primary indicator of toxicity since a dose-dependent increase in the fluoride content of the teeth and bone in both sexes was noted at doses ≥300 ppm. This disturbance in metabolism was reflected by hyperostosis of the cranium in males and females at doses ≥1500 ppm. These findings were consistently observed in carcinogenicity studies in rats.

IV. TOXICOLOGY

1. Metabolism

In a metabolism study in rats tolylfluanid was administered in single doses of 2 or 25 mg/kg of body weight, was readily absorbed and rapidly hydrolyzed within 48 hours. Absorption and excretion was independent of dose and sex. About 86 - 100% of the dose was recovered in 48 hours, with 56 - 80% of the dose being excreted in the urine, 12 - 36% in the feces, and ≤ 0.48% was found in the carcass. Urinary metabolite common to both sexes were dimethylaminosulfonylamino-benzoic acid (RNH 0166; 46 - 78%), and 4-methylamino-benzoic acid (RNH 0416; 3 - 6%). Fecal compounds identified were unchanged tolylfluanid (1 - 19%), dimethylaminosulfotoluidid (DMST; 5 - 8%), RNH 0166 (3 - 12%) and RNH 0416 (< 1%). The data indicate that tolylfluanid was hydrolyzed to DMST, which was then transformed into a major metabolite RNH 0166, which further demethylated to the minor metabolite, RNH 0416 (MRID No. 44285805).

Additional data (MRID Nos. 45302728 - 45302732) using labeled [dichlorofluoromethyl-^{14}C]-tolylfluanid and benzene ring showed that metabolic profile was dependent on the position of the label. With [dichlorofluoromethyl-^{14}C]-tolylfluanid labeling major urinary metabolite was thiazolidine-2-thione-4carbonic acid resulting from cleavage of the side chain and accounted for 73 - 74% and 50 - 63%, respectively by the iv and oral routes. Benzene ring label resulted in metabolite 4-(dimethylamino-sulfonylamino) benzoic acid which accounted for 90% of urinary metabolic activity and 70% of fecal radioactivity. The study with single oral dose of 2 or 20 mg/kg/day also supported the results of the main study (MRID No. 45302728).

2. Mutagenicity:

A series of five Ames assays (1 on the technical and 4 on various metabolites) were negative. One of the metabolites was tested and found to be negative in the mouse lymphoma forward gene mutation assay. However, the nonactivated parent compound induced reproducible increases in the mutation frequency (MF) of mouse lymphoma cells at severely cytotoxic (≤10% survival) concentrations and at moderately cytotoxic levels (≥17% survival). With S9 activation, dose-related increases in the MF were seen at moderately cytotoxic concentrations(≥18% survival) but tolylfluanid was negative for the induction of gene mutations in Chinese hamster ovary (CHO) cells and in CH lung fibroblasts (V79). There was also an *in vivo* gene mutation mouse spot test with clinical signs of toxicity in pregnant dams at 1750, 3500 and 7000 mg/kg. Results were negative but the study was classified as **Unacceptable** because there was no fetal toxicity. This study can be upgraded because the highest dose tested exceeded the limit dose.

Tolylfluanid was positive in both V79 cell and human lymphocyte cytogenetic assays. Dose-related positive responses were noted in human lymphocytes both with and without S9 activation at concentrations where ≥50% of the cells survived in separate trials. Positive results in V79 cells were only seen at a single dose (20 µg/mL). The most frequently observed chromosome aberration in the human lymphocyte assay was chromosome exchanges (types of aberrations were not specified in the V79 cell line).

In contrast to the positive *in vitro* cytogenetic studies, seven *in vivo* cytogenetic assays (bone marrow and spermatogonia in Chinese hamsters and mice) were available. All of these studies were negative but most were **Unacceptable**. There was, however, a negative mouse dominant lethal assay with 4000 and 8000 mg/kg which is currently listed as **Unacceptable** because positive control data were not included in the report. This study can be upgraded upon submission of the positive control data since the performing laboratory indicates that yearly positive controls were run for this assay system. An acceptable mouse bone marrow sister chromatid exchange (SCE) assay with levels up to the limit dose of 5000 mg/kg and an acceptable *in vitro* unscheduled DNA synthesis with levels up to cytotoxic concentrations were negative. No mutagenicity studies were found on Tolylfluanid in the open literature.

Results of mutagenicity studies indicate that tolylfluanid induced gene mutations and chromosomal aberrations in mammalian cells. For both genetic endpoints, positive results were generally seen in the presence and absence of S9 activation. These findings suggest a clastogenic effect since the positive result noted in the mouse lymphoma assay was not expressed in the *in vivo* test for gene mutations and could have arisen from chromosome aberrations. Tolylfluanid was negative in the dominant lethal assay and there was no indication of DNA damage either *in vitro* or *in vivo*. Similarly, no DNA adducts were found in the lung, liver or thyroid of male rats receiving tolylfluanid in diet up to 7500 ppm

in the ^{32}P-post labeling assay (MRID No. 45336212). Since all of the *in vivo* cytogenetic assays were negative and there was no indication of DNA reactivity either *in vivo* or *in vitro*, we conclude, that tolylfluanid has mutagenic potential, which is confined to *in vitro* test systems. Overall, the data do not suggest a mutagenic mode of action for thyroid tumors. Nevertheless, a data gap exists for a bone marrow cytogenetic assay (either micronucleus or chromosome aberrations). Until these data are submitted, no definitive conclusions can be reached regarding the potential of tolylfluanid to induce clastogenic effects in whole animal somatic cells. The summaries of the available mutagenicity studies are presented below.

Gene Mutation

870.5100 Bacterial gene mutation assay MRID 44241015 Acceptable	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to tolylfluanid (98.5%) at concentrations ranging from 1.25 to 5,000 µg/plate and were evaluated for mutagenicity at 5 - 160 µg/plate + S9 and at 1.25 to 40 µg/plate in the absence of S9. Tolylfluanid was cytotoxic to all strains at ≥ 8 µg/plate ± S9 and precipitated from solutions in all strains at 5000 µg/plate ± S9. **There were no reproducible, dose-related differences in the number of revertant colonies in any strain or dose over the background.** Positive controls induced appropriate response.
870.5100 Bacterial gene mutation assay MRID 44241016 Acceptable	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to WAK 5815, metabolite of tolylfluanid at concentrations in initial and repeat assays ranging from 16 to 5,000 µg/plate ± S9 (limit dose). **There was no evidence of toxicity or significant increase in mutant colonies over background in any of strains tested in either the initial or repeat mutagenicity assays.** Positive controls induced appropriate response.
870.5100 Bacterial gene mutation assay MRID 44241017 **Acceptable**	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were exposed to WAK 6550, metabolite of tolylfluanid at concentrations ranging from 16 to 5,000 µg/plate ± S9 (limit dose). **There were no reproducible, dose-related differences in the number of revertant colonies in any strain or dose over the background.** Positive controls induced appropriate response.
870.5100 Bacterial gene mutation assay MRID 44241018 **Acceptable**	*S. typhimurium* was exposed to WAK 6676, metabolite of tolylfluanid at concentrations ranging from 16 to 5,000 µg/plate ± S9 (limit dose). **There was no evidence of toxicity or significant increase in the mutant colonies over background in any strain tested.** Positive controls induced the appropriate responses in the corresponding strains and in the solvent controls were consistent with the expected ranges of revertant colonies for the strains used.

870.5100 Bacterial gene mutation assay MRID 44241019 Acceptable	*S. typhimurium* strains TA98, TA100, TA1536, and TA1537 were exposed to WAK 6698, metabolite of tolylfluanid at concentrations in initial assay from 16 to 5,000 µg/plate ± S9 (limit dose) and in the repeat assay from 16 to 1581 µg/plate ± S9. Tolylfluanid was cytotoxic at doses ≥158 µg/plate in the initial assay and 1,581 µg/plate in the repeat assay. **There was no evidence of significant increase in mutant colonies over background in any of strains tested in the initial or repeat mutagenicity assays.** Positive controls induced appropriate response.
870.5100 Bacterial gene mutation assay MRID 44241024 **Unacceptable**	*Saccharomyces cerevisiae* methionine auxotrophic strains S138 and S211∝ were exposed to tolylfluanid (99.1%). Strains S138 was tested at concentrations of 1 - 12.5, 10 - 50, or 50 - 200 µg/mL +S9 and 1 - 12.5, 10 - 100, or 10 - 50 µg/mL -S9. Strains S211∝ was tested at 1 - 12.5, 10 - 50, or 30 - 80 µg/mL +S9 and 1 - 12.5 µg/mL -S9. Tolylfluanid was tested to cytotoxic concentrations. Tolylfluanid showed no evidence of inducing methionine revertants in *Saccharomyces cerevisiae* strains ± S9. However, one of the tests (S211∝) was inadequate or inconsistent. Further, in the S9 activated assays, the positive controls did not elicit an adequate response, negating the test with S9 for both strains.
870.5300 *In vitro* mammalian cell gene mutation assay MRID 44241004 **Acceptable**	Mouse lymphoma cells were exposed to WAK 6698, metabolite of tolylfluanid at concentrations ranging from 1.95 to 1,000 µg/mL ± S9. The compound was tested up to cytotoxic concentrations in two independent assays (± S9). In the initial test concentrations ranged from 50 to 1,000 µg/mL ± S9. In the repeat assay concentrations ranged from 100 to 800 µg/mL -S9 and 200 to 700 µg/mL + S9. **Tolylfluanid metabolite was negative for inducing forward mutations at the TK locus in mouse L5178Y ± S9.** Positive control methyl methanosulfonate and 3-methylcholanthrene induced appropriate responses.
870.5300 *In vitro* mammalian cell gene mutation assay MRID 44241005 **Acceptable**	Chinese hamster ovary cells were exposed to tolylfluanid at concentrations ranging from 3.0 to 30.0 µg/mL + S9 and from 0.5 to 6.0 µg/mL - S9. These dose levels were selected based on a preliminary cytotoxicity study conducted at 0.5 to 250 µg/mL ± S9. **Tolylfluanid has been judged to be non-mutagenic ± S9.** Positive controls induced appropriate response ± S9.
870.5300 *In vitro* mammalian cell gene mutation assay MRID 44241008 & 45302707 **Acceptable**	Chinese hamster V79 cells were exposed to tolylfluanid (98.5%) at concentrations ranging from 300 to 3,000 ng/mL + S9 and from 4.0 to 40.0 ng/mL - S9. Cultures were tested to cytotoxic concentrations. **Tolylfluanid has been judged to be non-mutagenic ± S9.** Positive controls induced appropriate response ± S9.
870.5300 *In vitro* mammalian cell gene mutation assay MRID 44241025 **Acceptable**	Mouse lymphoma cells (L5178Y TK +/-) were exposed to tolylfluanid (99.1%) at concentrations ranging from 25 to 600 ng/mL -S9 and 50 - 12,500 ng/mL +S9. The compound was tested up to cytotoxic concentrations (± S9). **Tolylfluanid was positive for inducing forward mutations at the TK locus in mouse L5178Y ± S9.** Positive control ethylmethane sulfonate and 3-methylcholanthrene induced appropriate responses. Colony sizing was not performed.

Mouse spot test MRID 44241014 **Acceptable/non-guideline**	F1 pups from female $C_{57}BL_6/J$ mice exposed by oral gavage to tolylfluanid (98.4%) at concentration of 0, 1,750, 3,500 and 7,500 mg/kg did not show difference in incidence in relative spots between the treated and controls. Systemic toxicity was observed in dams at all doses. Mortality was observed at all doses; however there were no effects on reproductive parameters or differences in litter size. Positive controls showed a clear increase in spots in the progeny.

Cytogenetics

870.5375 *In vitro* mammalian chromosome aberration test MRID 44241020 **Acceptable**	Chinese hamster V79 cells were exposed to tolylfluanid (97.1 - 97.5%) at concentrations of 0.1, 0.5, and 1.0 µg/mL -S9 and 2, 10, and 20 µg/mL +S9. The test was conducted up to cytotoxic levels ± S9. **Tolylfluanid was weakly clastogenic in Chinese hamster V79 cells in the presence of S9 activation.** Positive control mitomycin and cyclophosphamide induced appropriate responses.
870.5375 *In vitro* mammalian chromosome aberration test MRID 44241012 & 45302712 **Acceptable**	Primary human lymphocytes were exposed to tolylfluanid (99.2%) at 0.1 to 10.0 µg/mL ± S9. Cytotoxicity was observed at concentrations of 1 to 10 µg/mL -S9 and 5 to 10 µg/mL +S9. Over the ranges tested clastogenic effects included increased incidences of metaphases with aberrations including gaps, metaphases excluding gaps, metaphases with exchanges, and metaphases with polyploidy. **Tolylfluanid is clastogenic both in the presence and in the absence of S9 activation.** Positive control mitomycin and endoxan induced appropriate responses.
870.5380 *In vivo* mammalian spermatogonia chromosomal aberration test MRID 44241011 **Unacceptable**	Tolylfluanid (93.1%) was administered to male Chinese hamsters at doses of 250 or 500 mg/kg/day by oral intubation for 2 days. No mortality or clinical signs were observed at either dose. No statistically significant increases in the frequency of chromosomal aberrations in spermatogonia were observed.

870.5380 *In vivo* mammalian spermatogonia chromosomal aberration test MRID 44241032 & 45302713 **Unacceptable**	Tolylfluanid (97.9%) was administered to male NMRI mice in cremaphor at doses of 500 or 1,500 mg/kg/day by oral intubation and were sacrificed 24 hours later. An addition groups of mice were administered 5,000 mg/kg/day and were sacrificed at 6, 24 and 48 hours. Clinical signs of toxicity and cytotoxicity to target cells were seen at 5,000 mg/kg/day. Tolylfluanid did not induce chromosomal aberrations in spermatogonia at any dose. Positive controls did not produce strong positive responses. Therefore, sensitivity of assay is questionable and the findings of the study are equivocal.
870.5385 Mammalian bone marrow chromosomal aberration test MRID 44241033 **Unacceptable**	*In vivo* bone marrow chromosomal aberration assay, Chinese hamsters were administered tolylfluanid (97.9%) at a single oral dose of 4,000 mg/kg/day and bone marrow harvested 12 hours after treatment. 3/10 animals died but no clinical signs. No cytotoxicity was observed at the dose tested. Positive controls induced appropriate response. Due to inadequate sampling time and no indication of presence of test material at target site, data are not valid for regulatory purposes.
870.5385 Mammalian bone marrow chromosomal aberration test MRID 44241010 & 45302716 **Unacceptable**	*In vivo* bone marrow chromosomal aberration assay, three groups of Chinese hamsters were administered tolylfluanid (99.7%) at a single oral dose of 4,000 mg/kg/day and bone marrow harvested 6, 24, and 48 hours after treatment. 3/10 animals died but no clinical signs. No clinical signs of toxicity were observed at the dose tested. Test results were erratic. Positive controls induced appropriate response. The study is inadequate since test samples were not analyzed and doses were not high enough to produce toxicity.
870.5395 Mammalian erythrocyte micronucleus assay MRID 44241009 **Unacceptable**	Tolylfluanid (93.4%) was administered to male NMRI mice in split doses of 250 or 500 mg/kg in 1% Cremaphor by oral intubation. The second dose administered 24 hours after the first dose. Bone marrow was harvested 6 hours following the 2nd dose. No clinical sings of toxicity was observed and was not toxic to the target tissue. Treatment with tolylfluanid did not induce micronucleated polychromatic erythrocytes. The study deficiencies include inadequate methods and methodology.
870.5450 Dominant lethal assay - mice MRID 44241013 **Unacceptable but upgradable with receipt of positive control data**	Male NMRI mice were orally exposed to tolylfluanid (98.8%) at doses of 4,000 or 8,000 mg/kg and mated sequentially to female mice did not induce variations in any dominant lethal parameters nor any reduced fertility. The study was inadequate and there were no positive control data.

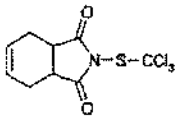
870.5915 *In vivo* Sister chromatid exchange assay MRIDs 44241031 & 45302722 **Acceptable**	Male and female NMRI mice dosed orally with Tolylfluanid at 0, 500, 1,670, and 5,000 mg/kg, and sacrificed at 24 hours and bone marrow cells were analyzed for sister chromatid exchange. Mortality occurred at 500 mg/kg and above. **Tolylfluanid did not induce sister chromatid exchange at any dose level.** Positive control cyclophosphamide responded appropriately.

Other Genotoxicity

870.5550 Unscheduled DNA synthesis UDS in mammalian cell culture MRID 44241003 **Acceptable**	In a UDS assay rat hepatocytes were exposed to tolylfluanid (98.7%) in ethanol at 2.5, 5.0, 10.0, 12.5, 15.0, 17.5, or 20 µg/mL for 16 - 24 hours. **Tolylfluanid did not induce UDS up to 15.0 µg/mL.** The 17.5 and 20 µg/mL doses were highly toxic. The positive control 2-acetylaminofluorene responded appropriately.

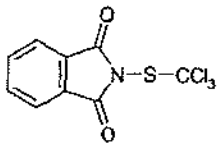
3. Structure-Activity Relationship

Tolylfluanid is structurally related to captan, folpet and captofol. However, the CARC noted that with the exception of few special circumstances, C-F bond is much more stable than C-Cl bond. Therefore, tolylfluanid should be a considerably weaker direct-acting alkylating agent than the analogs. This is consistent with the much weaker or marginal genotoxic activity of tolylfluanid. In addition, unlike tolylfluanid, captan and folpet induce tumors of the gastrointestinal tract The carcinogenic and mutagenic potential of these compounds is discussed below.



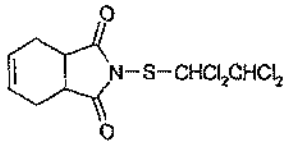
Captan

Captan (PC Code 081301; CAS No. 133-06-2), a dicarboximide, is classified as a **Group B2, Probable Human Carcinogen with a q_1*** based on statistically significant increases in combined renal cortical/tubular cell adenomas/carcinomas in male Charles River CD rats and an increase in the incidence of combined duodenal adenoma/polyps or adenocarcinomas in male and female $B_6C_3F_1$ mice. It was mutagenic in bacteria, eukaryotic microorganisms and mammalian cell cultures. **It gave a positive response for gene mutation and induced unscheduled UDS assay and chromosomal aberrations.**



Folpet

Folpet (PC Code 081601; CAS No. 133-07-3) is a structural analogue of Captan. **Folpet** is classified as a **Group B2, Probable Human Carcinogen with a Q_1*** based on statistically significant increases in adenomas and carcinomas of the duodenum in both sexes of CD-1 and B6C3F1 mice. It was mutagenic in *S. typhimurium* (his), *E. coli*, (WP2), mouse lymphoma (L5178Y), and in the sex-linked recessive lethal assay in Drosophila. Mutagenic activity was usually reduced upon addition of liver homogenates. **Folpet was negative in the rat bone marrow cytogentic and mouse somatic cell mutation assays.**



Captafol

Captafol *(PC Code 081701; CAS No. 2939-80-2)* is a structural analogue of Captan and Folpet. **Captafol** is classified as a **Group B2, Probable Human Carcinogen with a Q_1*** based on the increased incidence of lymphosarcomas and hemangiosarcomas in male and female mice and harderian gland adenomas in male CD-1 mice as well as combined renal adenomas/carcinomas in male Sprague-Dawley rats. Captafol is mutagenic in bacterial systems and mammalian cells in culture. Mutagenic activity is reduced or abolished in the presence of S-9, cysteine, glutathione, and when pre-incubated with blood, urine or plasma. **Captafol was negative in *in vivo* assays.**

h. Other studies (evidence desirable):

There is evidence that *in vivo* ^{32}P-postlabelling assay tolylfluanid at dose of 7500 ppm did not induce DNA adducts in rat liver, lung, and thyroid suggesting that tolylfluanid is unlikely to cause thyroid tumors in rats by mutagenic mode of action.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

Rats

1) In a subchronic toxicity study (MRID 44241006, 44285806 and 45302614), tolylfluanid technical (97.5% a.i., Lot #231282126) was administered to Wistar rats (10 or 20/sex/dose) by feeding at dose levels of 0, 300, 1650, or 9000 ppm (0, 20.1, 108.0, or 638.9 mg/kg/day for males; 0, 23.0, 131.0, or 736.1 mg/kg/day for females) for 13 weeks. After 13 weeks, 10 rats/sex/dose were sacrificed and necropsied. An additioual 10 rats/sex in the 0 and 9000 ppm treatment groups were maintained without treatment for 4 weeks to determine recovery, prior to sacrifice and necropsy.

In rats treated at 9000 ppm, clinical blood chemistry parameters associated with the liver and thyroid were significantly affected by treatment with tolylfluanid. At weeks 4 and/or 13, rats had lower aspartate aminotransferase (9-22%), alanine aminotransferase (24-44%), and alkaline phosphatase activities (14-21%), lower urea (14-18%) and higher cholesterol

(16-18%), thyroxine-binding capacity (TBC)(6-12%) and thyroid stimulating hormone (TSH)((35-38%) levels compared to the controls. Relative liver weights of males were 6% higher than the controls; no other changes in liver or thyroid weights or histology were observed. Mean body weight gains of both sexes were 9-10% lower than the controls at 13 weeks. At 1650 ppm, males had lower alanine aminotransferase and alkaline phosphatase activities compared to the controls. At 300 ppm, there were no apparent treatment-related responses. Following 4 weeks of recovery for the 9000 ppm treatment groups, the toxicological effects of tolylfluanid disappeared, with the exception of slightly elevated TBC levels in males compared to the controls. No treatment-related deaths occurred during the study, and there were no treatment-related differences in the appearance or behavior, ophthalmology, hematology, absolute organ weights, or gross or microscopic histology of the rats. No neoplastic tissue was observed. **The LOAEL for this study is 1650 ppm (108.0 mg/kg/day), based on changes in clinical blood chemistry associated with the liver and thyroid. The NOAEL is 300 ppm (20.1 mg/kg/day).** This study is classified as **Acceptable/Guideline.**

2) In a subchronic neurotoxicity study (MRIDs 44241007, 45302724 & 45302726), tolylfluanid technical (97.5% a.i., Batch # 231282126) was administered to Wistar rats (12/sex/dose) by feeding at dose levels of 0, 300, 1650, or 9000 ppm (0, 20, 109, or 620 mg/kg/day for males; 0, 25, 134, or 771 mg/kg/day for females) for 13 weeks. The rats were evaluated by functional observation battery and motor activity testing 1 week prior to treatment and during weeks 4, 8, and 13 of treatment.

No treatment-related neurotoxicological effects were observed at any treatment level. Body weight gains of rats in the 9000 ppm treatment groups were 7-8% lower than the controls at the termination of treatment, and mean food consumption was slightly (3-8%) higher. Females in the 1650 ppm treatment group had mean body weight gains 10% lower than the controls. No treatment-related deaths occurred during the study, and there were no treatment-related differences in the general appearance or behavior, ophthalmology, absolute or relative brain weights, or gross or microscopic histology of the rats. **The LOAEL is 1650 ppm (134 mg/kg/day) based on decreases in mean body weight gain in females. The NOAEL is 300 ppm (20 mg/kg/day.** This study is classified as **Acceptable/Guideline**

Dog

In a subchronic toxicity study (MRID 44247601), tolylfluanid (purity unspecified, Lot #1287) was administered to four beagle dogs/sex at dietary concentrations of 0, 400, 1000 or 3000 ppm (equivalent to 0, 8.24, 25.0 or 69.4 mg/kg/day for males and 0, 8.00, 23.1 or 67.2 mg/kg/day for females) for 13 weeks.

Male and female dogs in the 3000 ppm treatment group had lower body weight gains (58-

63%) and reduced food consumption (7-10%) compared to the controls, and liver abnormalities. Slightly intensified PAS reactions in liver cells were observed in 6/8 dogs compared to 2/8 controls. Age-related decreases in alkaline phosphatase activity over time were delayed compared to the controls; by week 13, mean group activities differed by 43-50%. Relative liver weights were increased 10-13% above controls. No treatment-related effects were observed at 1000 or 400 ppm. No animals died and no treatment-related differences in ophthalmology, hematology, urine parameters, absolute organ weights, or gross histopathology were observed. No neoplastic lesions were observed. **The LOAEL for this study is 3000 ppm (67.2 mg/kg/day), based on decreased body weight gains and changes in liver structure and function in both sexes. The NOAEL is 1000 ppm (23.1 mg/kg/day).** The study is classified as unacceptable but can be upgraded, provided the analytical data on the stability of the test substance in the diet are provided and found acceptable.

b) Chronic Toxicity

For treatment-related non-neoplastic changes in two rat studies and a mouse study refer to pages 7, 12 and 14, respectively).

Dog

1) In a chronic toxicity study (MRID 44241026), tolylfluanid (99.2% a.i., Lot #16002/82) was administered via capsules to four beagle dogs/sex/dose at dose levels of 0, 2.5 or 12.5 mg/kg/day for 52 weeks, or at 62.5 mg/kg/day for 33 weeks followed by 125 mg/kg/day for 19 weeks.

In dogs treated at 62.5 mg/kg/day, body weight gains at week 33 (final weeks at this dose level) were lower in males (50%) and females (11%) compared to the controls. After the dose rate was increased to 125 mg/kg/day (weeks 34-52), both sexes had decreased final body weights (0.97-2.55 kg) and lower body weight gains (37-58%) than the controls. The kidneys exhibited dilation of damaged tubules, epithelial flattening, focal hypertrophy and/or epithelial desquamation. Creatinine levels were elevated in both sexes. Urea was elevated in males. Glucose was detected in urine from several dogs. Expected age-induced decreases in alkaline phosphatase activity were delayed in both sexes. Alanine aminotransferase and glutaldehyde dehydrogenase activities were increased in 1/4 males and 4/4 females. The 12.5 and 2.5 mg/kg/day treatment groups did not exhibit treatment-related effects. There were no deaths. No treatment-related changes in appearance, behavior, food consumption, ophthalmology, hematology, N-demethylase activities, cytochrome P-450 concentrations, organ weights, or gross histopathology were observed. No neoplastic tissue was observed. **The LOAEL is 62.5 mg/kg body weight/day, based on decreased body weight gains in males treated at this dose level. The NOAEL is**

12.5 mg/kg body weight/day. This study is classified as **Acceptable/Guideline**

2) In another chronic oral toxicity study (MRID 45302624, 45302625 & 45336211), KUE 13183b (tolylfluanid; 96.7-98.6% a.i., batch/lot # 23182087) was administered to 4 beagle dogs/sex/dose in capsules at dose levels of 0, 5, 20, or 80 mg/kg/day for 52 weeks.

Treatment with up to 80 mg/kg/day did not adversely affect survival, clinical signs, food and water consumption, blood pressure measurements, pulse, ECG recordings, ophthalmoscopic findings, hematology or clinical chemistry parameters, urinalysis results, enzyme determinations in liver tissue, or gross or microscopic findings.

The differences in body weights observed in high-dose males are considered an adverse effect of treatment. Although the changes were not statistically significant, they were consistently biologically significant, with body weight gains for the high-dose males ranging from 72-75% of controls for the treatment intervals of weeks 1-13, 13-26, 26-53, and 1-53. Final body weights were 93% of controls (not significant). The decreases in body weight gains observed in this study are supported by the decreased overall body weight gain observed in high-dose males from a 52-week dose range-finding study in beagles (68% of historical control mean; high-dose dogs received 62.5 mg/kg bw for weeks 1-33 and 125.0 mg/kg bw for weeks 34-52; MRID 45336211). Female body weight gains were not affected by treatment.

The LOAEL is 80.0 mg/kg bw/day for male dogs based on decreased body weight gains and the NOAEL is 20.0 mg/kg bw/day. The NOAEL for females is 80.0 mg/kg bw/day and the LOAEL could not be determined. This study is classified as **Acceptable/Guideline.**

5. Mechanistic Studies

The results of 28- and 90-day feeding studies in rats indicate that tolylfluanid causes decrease in T3, T4 and increase in TSH levels in rats. In a ^{32}P-postlabelling assay, tolylfluanid did not form DNA adducts in lung, liver and thyroids. These findings support the nonmutagenic mode of action for the induction of thyroid tumors. Furthermore, 2-thiazolidinethione-4-carboxylic acid (TTCA), a metabolite of tolylfluanid, was shown to cause dose-dependent inhibition of the thyroid-peroxidase (TPO) activity in an *in vitro* assay using guinea pig cells. TPO mediated reactions are known to be involved with initial stages of thyroid hormone synthesis. TTCA behaves as a goitrogenic compound with potency equal to that of propylthiouracil (PTU), a known thionamide inhibitor of initial thyroid hormone syntheses. These studies collectively, suggest that metabolite 2-thiazolidinethione-4-carboxylic acid may be responsible for the increased incidence of thyroid tumors observed in the rat carcinogenicity studies. TTCA was a major metabolite of tolylfluanid in the rat metabolism study. The studies relevant to the mode of action of

thyroid tumors in rats are discussed below.

1) In a 90-day subchronic toxicity study, an increases in thyroxine-binding capacity (6%-12%) and TSH levels (35%-38%) as well as increased relative liver weights were noted in treated male rats. However, no histopathological changes in the liver and thyroid were seen. Refer to subchronic toxicity study (MRIDs 44241006, 44285806 and 45302614; p.22) for further details.

2) In a 28-day oral feeding study (MRID 45302615), tolylfluanid (purity 91%, batch/lot 160) was administered to 10 Wistar Bor:WIsW rats/sex/dose in the diet at dose levels of 0, 300, 1500, or 7500 ppm (equivalent to 0, 20.6, 119.3, or 677.9 mg/kg bw/day for males and 0, 22.1, 118.8, or 752.4 mg/kg bw/day for females).

The test material induced a treatment-related decrease in the body weights of male rats receiving ≥1500 ppm and in female rats receiving 7500 ppm of the test material. In addition, a treatment-related decrease in food efficiency was found in these dose groups. Treatment had no effect on plasma cholesterol and triglyceride, but did induce a slight decrease in circulating T4 (21%) in male rats receiving ≥1500 ppm test material and female rats that received 7500 ppm. No biologically significant differences in the circulating levels of T3 were found. TSH was statistically increased (168% to 425%) in high-dose male and female rats, but the biological significance was questionable. No significant treatment-related effects were noted on absolute or relative organ weights or histopathology. The thyroid parenchyma was pale in 9/10 high-dose male rats and 6/10 high-dose female rats, however there were no histological correlates and the significance is unknown.

The study results suggest, but do not confirm, that short-term treatment with the test material may induce effects that result in the formation of thyroid tumors. While circulating T3 and T4 levels were within historical control limits, the 2-4 fold increase of TSH suggests a perturbation of thyroid homeostasis. It is conceivable that the effects noted in this 28-day study could be a precursor to the induction of thyroid tumors during a 2-year study. However, the study had several deficiencies as follows: the absolute and relative liver weights were not provided and assays for hepatic enzyme activity associated with thyroxine excretion were not conducted. As a result the role of the liver in the disruption of thyroid hormone levels cannot be determined. This study is classified as **Acceptable/Non-guideline**.

3) In a ^{32}P-postlabelling assay for detection of adduct formation in lung, thyroid, and liver DNA in rats (MRID 45336212), groups of four male Wistar rats were administered tolylfluanid (Lot No. 231382087, 100.5%) in the diet at doses of 1500 or 7500 ppm for 21 days. 2-Acetylaminofluorene (2-AAF) was administered by gavage at 10 mg/kg/day for 7 days and served as a positive control for liver, lung, and thyroid DNA adducts. Benzidine was administered by gavage at 50 mg/kg/day for 7 days and served as a positive control for

lung DNA adducts. 2-Thiourea was administered by gavage at 17.5 mg/kg for 1 day and at 8.75 mg/kg/day for 6 days and served as a positive control for lung and thyroid DNA adducts. Finally, Dibenz[a,h]anthracene (DBA) was administered by gavage daily at 200 mg/kg/day for 7 days and served as a positive control for DNA adducts in the lungs. Olive oil (2% in the diet for 21 days) served as a solvent control.

There was no mortality during this study. However, the high dose tested was considered adequate based on decrease in body weight in rats administered 7500 ppm Tolylfluanid. **There was no evidence of DNA adduct formation in the liver, lung, or thyroid of rats exposed to Tolylfluanid.** The positive and solvent controls induced the appropriate response. This study is classified as **Acceptable/Nonguideline.**

4) A series of *in vitro* assays (MRID 45302634) were conducted to investigate whether inhibition of thyroid hormone synthesis by tolylfluanid or its metabolite 2-thiazolidinethione-4-carboxylic acid (TTCA) was responsible for the increased incidence of thyroid tumors observed in earlier studies. The results of these studies show that the thionamide TTCA behaves as a reversible inhibitor of thyroid peroxidase (TPO)-mediated reactions involved with the initial stages of thyroid hormone synthesis. This was shown by the dose-dependent decrease in formation of reactive iodine and the interference of the nonenzymatic and TPO-mediated iodination of L-tyrosine, and by TPO-mediated metabolism of TTCA. In the latter reaction, TTCA did not interfere with tyrosine iodination when the concentration in the reaction mixture fell below a certain concentration. Therefore, TTCA, unlike tolylfluanid, behaves as a goitrogenic compound with a potency approximately equal to propylthiouracil (PTU), a known thionamide inhibitor of initial thyroid hormone synthesis.

This *in vitro* study is **Acceptable/Non-guideline** and provides information on the inhibition of the initial stage of thyroid hormone synthesis.

Weight-of-Evidence Considerations in the Determination of the Mode of Action for Thyroid Follicular Cell Tumors in Rats

The CARC considered the following toxicology data in determining the carcinogenic potential and the method for the quantification of human cancer risk of tolylfluanid:

1) In the 1982 combined chronic toxicity and carcinogenicity study in rats, administration of tolylfluanid in the diet at 0, 300, 1500 or 7500 ppm (0, 20, 80, or 430 mg/kg/day for males and 0, 20, 110, or 580 for females) for 2 years was associated with statistically significantly increasing trends for combined thyroid follicular cell adenomas/carcinomas in both sexes. In addition, in females there was a statistically significant increasing trend for thyroid follicular cell adenomas.

The following evidence of liver pathology was observed: a) increased relative liver weights in high-dose male and female rats, b) increased incidence of liver necrosis in high-dose males, c) increased incidence of bile duct proliferation in high-dose females.

The evidence of thyroid pathology observed at 7500 ppm included an increased incidence of thyroid follicular cysts in high-dose females. Hyperplasia was not noted. No thyroid hormone measurements were made. In addition, there was increased incidence of hyperostosis of the cranium, and sternum in high-dose males and females.

The dosing at the highest dose was considered to be adequate and not excessive for assessing the carcinogenic potential of tolylfluanid based on decrease in body weight gain in the high-dose males and females throughout the study.

2) In the 1996 combined chronic toxicity and carcinogenicity study in rats, administration of tolylfluanid in the diet at 0, 60, 300, 1500 or 7500 ppm (0, 3.6, 18.1, 90.1 or 504.2 mg/kg/day for males and 0, 4.2, 21.1, 105.2 or 584.4 for females) for 2 years was associated with significantly increasing trends and significant differences in the pair-wise comparisons of the high-dose group with controls for thyroid follicular cell adenomas and combined adenomas/carcinomas in both sexes.

The following evidence of liver pathology was observed at 7500 ppm: increased relative liver weights and hepatocellular alterations; however, these changes were considered equivocal.

The evidence of thyroid pathology observed at 7500 ppm included an increased incidence of thyroid follicular hyperplasia in both sexes. No thyroid hormone measurements were made.

In addition, increased incidence of bone mineralization was evidenced by hyperostosis of the cranium in high-dose males and females. Also there was an increase in fluoride deposition in the teeth and bone in both sexes. In light of the systemic effects observed, dosing was considered to be adequate and not excessive for assessing the carcinogenic potential of tolylfluanid in the rat. The body weight gain was decreased throughout the study for the high-dose males and females.

3) In the mouse carcinogenicity study, administration of tolylfluanid was not associated with a statistically significant increase in the incidence of any neoplasms at any treatment level.

Dosing was considered adequate and not excessive based on decreases in body weights and body weight gains in high-dose males. Disturbance in fluoride metabolism was evidenced as: a) dose-dependent increased fluoride content of teeth and bones in both sexes at doses 300 ppm and above, and b) hyperostosis of cranium in both sexes at doses 1500 ppm and above. Furthermore, the highest doses employed was the limit dose (7500 ppm; *HDT*) for this species.

4) In chronic toxicity study in dogs administration of tolylfluanid at dose levels of 0, 2.5 or 12.5 mg/kg/day for 52 weeks, or at 62.5 mg/kg/day for 33 weeks followed by 125 mg/kg/day for 19 weeks; or 0, 5, 20, or 80 mg/kg/day for 52 weeks in capsules was associated with decreased body weights and body weight gains in males at 62.5 - 80 mg/kg/day. Liver and thyroid weights were not effected. Cytochrome P-450 concentrations or N-demethylase activities were not elevated.

5) In a subchronic feeding study in rats administration of tolylfluanid at 0, 300, 1650, or 9000 ppm in the diet for 13 weeks was associated with changes clinical chemistry of the liver and thyroid at 1650 ppm. At 9000 ppm, there was an increase in the thyroxin-binding-capacity (TBC) and thyroid stimulating hormone level. Following a 4 week recovery period the toxicological effects of tolylfluanid disappeared, with exception of the TBC levels which were slightly elevated in males. Liver weights were increased 6% in the high-dose males. Thyroid weights were not affected. No treatment-related histopathological findings were observed in liver and thyroid.

6) In a metabolism study rats given [dichlorofluoro-methyl-^{14}C]-tolylfluanid, the major metabolite was thiazolidine-2-thione-4-carbonic acid resulting from cleavage of side chain. This metabolite accounted for 50 - 74% of the dose administered via intravenously or orally. This metabolite was tested in a *in vitro* goitrogenic assay. The metabolite 2-thiazolidinethione-4-carboxylic acid (TTCA) was a dose-dependant reversible inhibitor of thyroid peroxidase (TPO)-mediated reactions involved with initial stages of thyroid hormone synthesis. Tolylfluanid did not inhibit the reaction. This suggests that TTCA, unlike tolylfluanid, behaves as goitrogenic compound with a potency approximately equal to propylthiouracil (PUT), a known thionamide inhibitor of initial thyroid hormone synthesis. Perturbence of thyroid homeostasis could result in thyroid tumors.

7) In a ^{32}P-postlabelling assay tolylfluanid did not form DNA adducts in rat lung, liver and thyroids, suggesting that the mechanism does not apply to tolylfluanid. DNA adducts are precursors for thyroid tumorigenesis.

8) In a special thyroid function study Tolylfuanid was administered to Wistar rats at dietary concentrations of 0, 300, 1500 , or 7500 ppm, for 28-days. At 1500 ppm,

there was a slight decrease in circulating T4 in male rats receiving ≥ 1500 ppm and female rats receiving 7500 ppm. No biologically significant differences in the circulating levels of T3 were found. The circulating levels of TSH were elevated 2 to 4 fold. This suggests that short-term treatment with tolylfluanid could cause hormone perturbation of thyroid homeostasis, which may serve as a precursor event for the induction of thyroid tumors in long-term studies. Pale thyroid parenchyma was noted in high-dose males and females. Thyroid weights and histopathology were unaffected by the treatment. Liver weights were not measured.

Consideration of the Use of the Threshold Model for Tolylfluanid

The Committee was asked to consider whether use of a non-linear approach to quantification of thyroid neoplasms was appropriate for tolylfluanid based on the framework presented in the Agency's Policy Document entitled "Assessment of Thyroid Follicular Cell Tumors", March 1998 (EPA/630/R-97/002).

The policy document states that:

"Tumors of the thyroid gland follicular cells are fairly common in chronic studies of chemicals in rodents. Experimental evidence indicates that the *mode of action* for these rodent thyroid tumors involves (a) changes in the DNA of thyroid cells with the generation of mutations, (b) disruption of thyroid-pituitary functioning, or (c) a combination of the two. The only verified cause of human thyroid cancer is ionizing radiation, a *mutagenic* insult to which children are more sensitive than adults.

"Treatments of rodents that cause thyroid-pituitary disruption result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and, finally, tumors of the thyroid. In some cases, there is also an increase in tumors of the pituitary cells that produce TSH. Cessation of treatment early in the process before tumor development results in reversal of processes back towards normal."

When assessing tumors of the thyroid, "For those cases where thyroid tumors arise from chemically induced disturbances in thyroid-pituitary functioning, tumors are considered to be secondary to the adverse effects on the thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses where there is no effect on thyroid-pituitary homeostasis. Generally, homeostasis is considered to apply when serum T4, T3 and TSH levels and thyroid and pituitary morphology and growth are within their normal limits."

In the Science Policy Guidance section of this document, factors that should be considered in making this determination are discussed.

"Most of the focus in implementing this policy is devoted to answering the following questions:(1) Does an agent that shows thyroid carcinogenic effects have antithyroid activity? (2) Can modes of action other than thyroid-pituitary carcinogenic effects have antithyroid activity? (3) How can one express thyroid dose-response relationships?" The occurrence of tumors in tissues other than the thyroid is also considered in determining mechanism of carcinogenesis.

Consideration of whether the thyroid tumors associated with administration of tolylfluanid can be attributed to disruption of the thyroid-pituitary hormonal balance (antithyroid activity). In addressing this point, the Science Policy lists eight areas of inquiry for evidence demonstrating antithyroid activity (for additional details on the results described below, see individual study summaries presented earlier in this document for carcinogenicity and mechanistic studies):

a. Increases in cellular growth *in vivo* (evidence required):

In the chronic toxicity/carcinogenicity study in Wistar rats, treatment with tolylfluanid resulted in an increased incidence of thyroid follicular hyperplasia at dietary concentrations of 504 mg/kg/day in males and 584 mg/kg/day females, the highest dose tested in both sexes. In a second chronic toxicity/carcinogenicity study, follicular cysts were noted in females at 580 mg/kg/day. No increase in thyroid gland weights was noted at any dose level in chronic and subchronic toxicity studies in rats and dogs or in special mode of action studies in rats.

b. Hormone changes (e.g., reduced thyroid hormones T3, T4 and increased TSH; evidence required):

In the subchronic toxicity study, rats treated at 639 mg/kg/day tolylfluanid had increased circulating T3 and T4 levels in males, and increased thyroid binding capacity (TBC) and thyroid stimulating hormone (TSH) levels in male and female rats at week 13 compared to controls. Following a 4 week recovery, the T3, T4, and TSH levels returned to normal levels, except TBC which was slightly elevated. Relative liver weights were slightly higher; however no adverse histopathology was observed for liver and thyroid. In a special thyroid function study, rats treated with 678/752 mg/kg/day tolylfluanid showed an equivocal decrease in circulating T3 and T4 levels. TSH levels increased 2 fold. T3 and T4 hormone levels were within the historical control levels provided in the study. Pale thyroid parenchyma was observed in high-dose males and females. No differences were observed in liver or thyroid organ weights or histopathology.

c. Site of action (intra thyroidal, peripheral tissues, liver or other sites; evidence required):

The available toxicology data suggest that the primary site of action may be the liver, and to a lesser extent, the thyroid. The chronic and subchronic studies demonstrate that tolylfluanid affected relative liver weights resulting in increases of 6 - 24%, compared to the controls. It is anticipated that increased liver weights might induce cellular hypertrophy, resulting in microsomal enzyme induction (not measured), however, cellular hypertrophy was not observed in any of the studies. Enhanced hepatic metabolism of the thyroid hormones leads to decreased serum levels of T3 and T4. The decreased serum levels of these hormones causes an increased release of TSH. The higher serum TSH level in turn causes thyroid hypertrophy and hyperplasia leading to thyroid gland neoplasia. Fluctuating T3 and T4 levels and elevated TSH levels observed in the 13 week feeding and special thyroid function studies did not provide convincing evidence of a perturbed thyroid-pituitary homeostasis which resulted in tumorigenesis. Furthermore, there was no increase in the thyroid weights nor was there any histological correlate to support the thyroid homeostasis phenomenon.

In chronic dog studies, cytochrome P-450 concentrations and N-demethylase activity were affected at doses 62.5 - 80 mg/kg/day.

d. Dose correlations (evidence required):

The available data (2-year rat combined chronic toxicity and carcinogenicity study , subchronic feeding rat study, and special thyroid function study) indicate that the increase in thyroid follicular cell tumors may be correlated with a perturbence of thyroid hormone levels and hyperplasia in both sexes. Increases in thyroid tumors were only observed at high-dose levels causing these effects (7500 ppm).

e. Reversibility (evidence required):

The subchronic toxicity study demonstrated that after the 4 week recovery phase, there were no treatment-related differences in T3, T4 and TSH levels, except for slightly elevated (7%) thyroid binding capacity (TBC) in 9000 ppm (639 mg/kg/day) males. Relative liver weights were elevated (6%). Significantly elevated enzymes levels (SGPT, SGOT and alkaline phosphatase) were not considered biologically relevant since the differences were within the historical ranges. No other changes in the liver or thyroid weights or histology were observed.

f. Lesion progression (evidence desirable):

No evidence exists for lesion progression from the subchronic or special studies. In the rat

subchronic feeding study, no changes in the liver and thyroid weights or histology were observed. In a special thyroid function study, no treatment-related effects were noted on the absolute or relative organ weights or histology. Liver weights were not measured in this study. No indication of thyroid gland follicular cell hypertrophy or hyperplasia were seen in other subchronic toxicity or special studies.

Some evidence exists for lesion progression in the chronic studies. In rat 2 year chronic toxicity/carcinogenicity studies, increased incidence of thyroid follicular cell hyperplasia in males in one study and follicular cysts in females in another study were seen, both at 7500 ppm. In the1982 study, male and female rats had a significant increasing trend for combined thyroid follicular cell adenomas/carcinomas. Female rats also had significant increasing trends for thyroid follicular cell adenomas. In the 1996 study, male and female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls, for thyroid follicular cell adenomas and combined adenomas/carcinomas.

h. Structure-activity analysis (evidence desirable):

Captan, folpet, and captafol are structural analogues of tolylfluanid; however, the tumors resulting from the exposure differ.

Consideration of dose-response:

In the chronic toxicity/carcinogenicity studies in rats, thyroid effects were observed at the same dosages at which increases in thyroid tumors were observed at 7500 ppm (follicular cell hyperplasia in males -504 mg/kg/day and females - 584 mg/kg/day). Liver toxicity such as necrosis in high-dose males and bile duct proliferation in high-dose females were observed. Changes in liver function parameters such as increases in absolute and relative liver weights and histopathology indicative of cell hypertrophy or hyperplasia were suggestive of toxicity but not conclusive. The special studies provided evidence for perturbation of thyroid-pituitary homeostasis in the absence of any evidence of liver involvement (liver effects not measured). Equivocal liver enlargement might have resulted in enhanced hepatic metabolism of thyroid hormones leading to decreased/increased serum levels of T3 and T4. The increased/decreased T3 and T4 levels observed in the special studies do not reflect the pathophysiology of perturbed hormonal homeostasis. In reality decreased serum levels of these hormones causes an increased release of TSH and higher TSH levels which in turn cause thyroid hyperplasia.

Conclusions: Based on the overall judgement of the 8 sets of data evaluating evidence for thyroid activity, the CARC concluded that there are insufficient data to determine whether there is evidence that the tolylfluanid-induced thyroid tumors in the rat are associated with a disruption in the thyroid-pituitary homeostasis. In addition to the above data sets, the

statistically significant increase in the incidence of thyroid tumors in rats above the concurrent and historical control values in both sexes and in both studies was also considered in evaluating the carcinogenic potential of tolylfluanid.

Supporting Data

A series of *in vitro* assays showed that 2-thiazolidinethione-4-carboxylic acid, the metabolite of tolylfluanid, reversibly inhibits the thyroid-peroxidase (TPO)-mediated reactions in a dose-related fashion. These reactions are involved with initial stages of thyroid hormone synthesis.

Data Considered Insufficient

Refer to items listed under sections a, b, c, e, and f above.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

- Based on the results of two rat studies the CARC concluded that tolylfluanid was carcinogenic in male and female rats because in the 1982 study a treatment-related statistically significant increasing trend was noted at the high dose (7500 ppm) for thyroid follicular cell adenomas ($p \leq 0.05$) in females and combined thyroid follicular cell adenomas/carcinomas in both males ($p \leq 0.05$)and females ($p \leq 0.01$). Although these increases were not statistically significant by pair-wise comparisons with the controls, the increased incidences of thyroid follicular cell carcinomas as well as combined thyroid follicular cell adenomas/carcinomas in high-dose males and females and thyroid follicular cell adenomas in high-dose females were above the concurrent values and were either towards the high end or exceeded the respective historical control (HC) range as shown below.

  Males: carcinomas: 1/49, 2% vs 0/46, 0% in controls; HC: 0%-4%; mean:0.7%;
  combined adenomas/carcinomas: 3/49, 6% vs 0/46, 0% in controls; HC range 0%-4%; mean:0.7%;
  Females:adenomas: 3/50, 6% vs 0/50, 0% in controls; HC range: 0%-4%; mean:0.7%;
  carcinomas: 1/50, 2% vs 0/50, 0% in controls; HC: 0%;
  combined : 4/50, 8% vs 0/50, 0% in controls; HC range: 0%-4%; mean:0.7%.

Additionally in female rats, there was a statistically significant increasing trend ($p \leq <0.05$) for granulosa cell tumors of the ovary; however, no statistically significant increase by pair-wise comparison with controls was noted. The incidences of benign granulosa-theca cell tumors of the ovary tumors at 300, 1500 and 7500 ppm dose groups were 1/50, 2%; 1/50, 2% and 5/50, 10% vs 1/50, 2% in controls. The incidence at the high dose was within the historical control range (performing laboratory: 0%-8%, mean 1.2%; Bomhard and Rinke; 1994: 0%-8.3%, mean: 3%). In addition, the high dose females had increase in the incidence of malignant stromal sarcomas of the uterus (2/50, 4% vs 0/50, 0% in controls) but the incidence was within the historical control ranges (performing laboratory: 0%-4%, mean: 0.4%; Bomhard.and Rinke; 1994: 0%-6%, 0.6%). There were also increases in the incidence of uterine adenocarcinomas, carcinomas and combined adenocarcinomas/carcinomas in all dose groups. The incidences in the 300, 1500 and 7500 ppm dose groups were as follows: adenocarcinomas: 6/50, 12%; 9/50, 18% and 8/50, 16% vs 3/50, 6% in controls; carcinomas: 2/50, 4%; 2/50, 4% and 2/50, 4% vs 0/50, 0% in controls; combined adenocarcinomas/carcinomas: 8/50, 6%; 11/50, 22% and 10/50, 20% vs 3/50, 6% in controls. The incidence of combined adenocarcinomas/carcinomas in mid- and high-dose females was statistically significant by pair-wise comparison with the control and within the historical control range of the performing laboratory (0%-24%; mean 4.6%) and towards the high end of the historical controls values reported by Bomhard and Rinke; 1994 (0%-20%; 8%). These findings were not confirmed in the 1996 study and in the absence of dose-response and a statistically significant increasing trend, they do not add to the overall evidence of carcinogenicity of tolylfluanid. The highest dose was considered to be adequate and not excessive based on decrease in body weight gain (12-16%), decreased food consumption, bile duct proliferation, increased incidence of thyroid follicular cysts, and hyperostosis of cranium; the histopathological changes such as liver necrosis was observed in few high dose animals (5/38 vs 0/40 in controls). The survival of the animals was unaffected by the treatment.

- The findings of thyroid tumors were reproducible in the 1996 rat study. In male and female rats, there was a statistically significant increasing trend ($p<0.01$) in the incidence of thyroid follicular cell adenomas and combined adenomas/carcinomas with increasing dose. In addition, a statistically significant increase by pair-wise comparison with the controls was also noted for thyroid follicular cell adenomas ($p \leq 0.05$) and combined adenomas/carcinomas ($p \leq 0.05$) in high-dose (7500 ppm) males and females. The increases in these tumors exceeded the concurrent control values and were either towards the high-end or exceeded the historical control range. The incidences of combined follicular cell adenomas/carcinomas in both male and female rats were driven by the adenomas. The incidences of these tumors in the high-dose groups were as follows:

Males: adenomas: 5/50, 10% vs 0/49, 0% in controls; HC mean: 1.2%
carcinomas: 1/50, 2% vs 0/49, 0% in controls; HC: 0%;
combined adenomas/carcinomas: 6/50, 12% vs 0/49, 0% in controls; HC mean: 1.2%
Females: adenomas: 5/46, 11% vs 0/49, 0% in controls; HC range: 0%-4%; mean:0.7%;
combined : 5/46, 11% vs 0/49, 0% in controls; HC range: 0%-4%; mean: 0.7%

The dosing was considered to be adequate and not excessive based on decrease in body weight gain, hepatocellular alterations (vacuolation, focal fatty changes etc.) and thyroid follicular cell hyperplasia in the mid- and high-dose males and females. The survival of the animals was unaffected by the treatment. **The CARC concluded that the thyroid tumors in the high-dose male and female rats were treatment-related.**

- The CARC concluded that tolylfluanid was not carcinogenic to male and female mice. In the mouse carcinogenicity study, administration of tolylfluanid in the diet was not associated with a significant increase in any type of tumors in mice. The *histiocytic* sarcomas in female mice occurred in all dose groups. The incidences were 5/50, 4/50, 2/50 and 6/50 at 60, 300, 1500 and 7500 ppm, respectively, vs 4/50 in controls. Because of lack of dose-response these tumors were not considered to be treatment-related. The dosing was considered to be adequate and not excessive based on decrease in body weight gain and an interference in the fluoride metabolism. The survival of the animals was not affected by the treatment.

2. Mutagenicity:

- The studies submitted by the registrant indicate that tolylfluanid induces gene mutations and chromosomal aberrations in mammalian cells in the presence and absence of S9 activation. Tolylfluanid was negative in the dominant lethal assay and there was no indication of DNA damage either *in vitro* or *in vivo*. Similarly, no DNA adducts were found in the lung, liver or thyroid of male rats receiving tolylfluanid in diet up to 7500 ppm in the ^{32}P-post labeling assay (MRID No. 45336212). Since all of the *in vivo* cytogenetic assays were negative and there was no indication of DNA reactivity either *in vivo* or *in vitro*, the CARC concluded, that the mutagenic potential of tolylfluanid is confined to *in vitro* test systems. Overall, the data do not suggest a mutagenic mode of action for thyroid tumors. However, the Committee determined that a bone marrow cytogenetic assay (either micronucleus or chromosome aberration test) is required in order to determine the potential of tolylfluanid to induce clastogenic effects in whole animal somatic cells.

3. Mode of Action:

- *In vitro* and *in vivo* mechanistic studies were conducted to determine whether thyroid tumors in rats were caused by the antithyroid activity of tolylfluanid. There was no increase in thyroid gland weight indicative of increased cellular growth *in vivo* noted at any dose level in chronic and subchronic toxicity studies in rats. In a 90-day subchronic rat study, there was no increase in thyroid weights nor were there any histopathological correlates to support the perturbation of thyroid-pituitary homeostasis. Relative liver weights were slightly elevated (6%). Enzymes levels (SGPT, SGOT and alkaline phosphatase), although significantly elevated, were within the historical ranges; no other changes in the liver or thyroid weights or histology were observed. There was no evidence for thyroid lesion progression from the subchronic or special studies. In a 28-day rat study, the increase in TSH levels was not biologically significant and the T3 and T4 hormone levels were within the historical control levels. The liver weights were not provided and assays for hepatic enzyme activity associated with thyroxine excretion were not conducted. 2-thiazolidinethione-4-carboxylic acid (TTCA), a metabolite of tolylfluanid, was shown to cause dose-dependent inhibition of the thyroid-peroxidase (TPO) activity in an *in vitro* assay in guinea pig cells. TPO mediated reactions are known to be involved with initial stages of thyroid hormone synthesis. TTCA was a major metabolite of tolylfluanid in the rat metabolism study.

Consideration of the Use of the Non-linear Extrapolation Approach for Tolylfluanid

When evaluating tolylfluanid, the Committee considered the possibility of using the non-linear extrapolation approach for thyroid neoplasms and the factors (as stated in the Science Policy Guidance; EPA, 1988b) in making the determination of whether the neoplasms are due to thyroid-pituitary imbalance. These include increases in cellular growth *in vivo*; hormone changes; site of action; dose correlations; reversibility; lesion progression; structure-activity analysis and other studies. The Committee also gave consideration to the extent to which genotoxicity may account for the observed tumor effects, the dose-response and the occurrence of tumors in other tissues in addition to the thyroid.

Based on the overall judgement of the 8 sets of data evaluating evidence for thyroid activity, the CARC concluded that the available data are insufficient to determine whether there is evidence that the rat thyroid tumors associated with administration of tolylfluanid are due to **a disruption in the thyroid-pituitary homeostasis.** In addition to evidence supporting disruption of the thyroid-pituitary homeostasis, the following factors were also considered in evaluating the carcinogenic potential of tolylfluanid: (1) the incidence of thyroid tumors in rats was statistically significantly increased above concurrent controls and above the historical control range in males and in females at 7500 ppm. 2-thiazolidinethione-4-carboxylic acid (TTCA), a metabolite of tolylfluanid, was shown to

cause dose-dependent inhibition of the thyroid-peroxidase (TPO) activity in an *in vitro* assay using guinea pig cells. TTCA was found to be a major metabolite of tolylfluanid in the rat metabolism study; (2) tolylfluanid did not demonstrate mutagenic potential in Ames gene mutation assays, chromosomal aberrations *in vivo*, and UDS assay. No DNA adducts were found in the liver, thyroid and lung of treated rats adducts but positive evidence of mutagenicity was noted in *in vitro* mammalian gene mutation and chromosomal aberration assays; (3) the structurally related compounds, captan, Folpet and captafol, did not induce thyroid tumors. Instead these compounds induced tumors of the gastrointestinal tract in rats. The weight-of-the evidence also indicates that tolylfluanid is not a strong mutagen although it has been shown in some studies to be weakly mutagenic. However, this information alone is NOT supportive of a non-linear mode of action for thyroid tumors. It is unclear whether, similar to other antithyroid agents in rodents tolylfluanid acts indirectly by causing sustained elevations in serum TSH levels associated with the development of thyroid carcinogenesis as oppose to rodent thyroid carcinogens that are directly DNA reactive. When considered together, the available information does not support the non-linear mode of action of interference with thyroid-pituitary homeostasis.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

There was a split opinion among the Committee members. In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified tolylfluanid into the category **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence consideration:

1. Thyroid tumors were seen in both sexes of rat and were reproducible in the 1996 study.

2. The relevance of the observed tumors to human exposure cannot be discounted.

3. The weight-of-the-evidence does not support the mutagenic mode of action for the induction of thyroid tumors in rats.

4. Data are not adequate to support an alternative mode of action for the thyroid tumor induction.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee made the following recommendations:

- For human cancer risk assessment, the linear low-dose extrapolation approach was recommended. The use of the linear approach is supported by lack of confirmation

that tolylfluanid causes thyroid tumors in rats by interference with thyroid-pituitary homeostasis.

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